

THE $^{11}\text{C}/^{14}\text{C}$ KINETIC ISOTOPE EFFECT METHOD. THE $^{11}\text{C}/^{14}\text{C}$ KINETIC ISOTOPE EFFECT IN THE $\text{S}_{\text{N}}2$ REACTION OF *N,N*-DIMETHYL-*p*-TOLUIDINE WITH LABELLED METHYL IODIDE

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Methodological aspects of the determination of $^{11}\text{C}/^{14}\text{C}$ kinetic isotope effects (KIEs) were investigated using the reaction of *N,N*-dimethyl-*p*-toluidine with labelled methyl iodide in methanol at 30 °C as model system. The primary ^{11}C KIE was determined to be 1.202 ± 0.008 for this reaction. The isotope effect was determined by a one-pot technique based on liquid chromatographic fractionation of the reactant and product followed by liquid scintillation counting of the ^{11}C and ^{14}C radioactivity in these fractions. The KIE value obtained agrees well with the value predicted from a previously determined $^{12}\text{C}/^{14}\text{C}$ KIE for the same reaction. Theoretical model calculations of both carbon KIEs were performed using the BEBOVIB IV program. Transition-state models ranging from reactant- to product-like were employed in the calculations, and three different reaction coordinate models with more or less pronounced Walden inversion of the methyl hydrogens were investigated.

INTRODUCTION

Kinetic isotope effects (KIEs) provide one of the most useful methods for the elucidation of organic and enzymatic reaction mechanisms. KIEs give information on which reaction step is rate limiting and, in combination with theoretical model calculations, a possible transition-state structure can be assigned.¹

Recently the first results of the $^{11}\text{C}/^{14}\text{C}$ KIE for the methylation of *N,N*-dimethyl-*p*-toluidine were presented in a short communication.² In the $^{11}\text{C}/^{14}\text{C}$ KIE method we utilize the fact that the radionuclides ^{11}C and ^{14}C have half-lives of 20.34 min and 5730 years, respectively. The $^{11}\text{C}/^{14}\text{C}$ KIE method is a one-pot procedure based on high-performance liquid chromatographic (HPLC) fractionation of the reactant and product of the reaction being studied. The ^{11}C and ^{14}C radioactivity of the fractions is then measured by liquid scintillation counting.

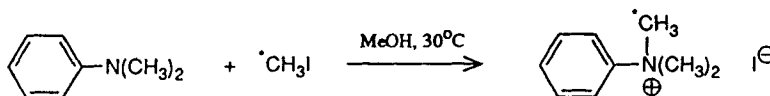
There are several advantages of combining ^{11}C and ^{14}C in KIE studies, e.g. a large mass range of carbon isotopes is used, resulting in a large isotope effect, and since both isotopes are radioactive they can be measured with high precision and accuracy. One-pot kinetic procedures are easily adopted, thus eliminating

inter-experimental errors, and in the key step, i.e. the HPLC fractionation, it is of little importance that the reactant and product fractions are free from impurities, as long as they are radioactively pure. Introducing ^{11}C makes four isotopes of carbon available for KIE studies. Thus experimental tests of theoretical predictions concerning the relative strengths of different carbon KIEs can be performed.³ The obvious major drawback to using ^{11}C are the restrictions imposed by its short half-life and the need for facilities for the production of the radionuclide ^{11}C close to the chemical laboratory.

The $^{11}\text{C}/^{14}\text{C}$ KIE method may seem very exclusive, but the increasing use of positron emission tomography (PET) in medical diagnoses and biomedical research has led to an increase in the number of accelerators available and the development of new methods for synthesis of ^{11}C -labelled molecules.⁴ In many syntheses of ^{11}C -labelled molecules enzymes are used in the synthetic route,⁵ to perform rapid and stereospecific reactions. Today a large number of synthetic routes for ^{11}C labelling are available and many molecules can be labelled.

Earlier, Buist and Bender⁶ determined the $^{12}\text{C}/^{14}\text{C}$ KIE for the reaction of *N,N*-dimethyl-*p*-toluidine with methyl iodide in methanol at 48.5 °C to be 1.117 ± 0.011 . The methylation reaction is clean and

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Scheme 1. * denotes the labelling position

can be adopted for $^{11}\text{C}/^{14}\text{C}$ isotope effect studies. This present paper gives a full experimental account of the determination of k_{11}/k_{14} , according to Scheme 1, of the methylation reaction at 30°C .

In this work, methodological aspects of the determination of $^{11}\text{C}/^{14}\text{C}$ KIEs were investigated using the title reaction as a model system. The relative strengths of the $^{12}\text{C}/^{14}\text{C}$ and $^{11}\text{C}/^{14}\text{C}$ KIEs were compared with a prediction (a derivation of the relationship between $^{12}\text{C}/^{14}\text{C}$ and $^{12}\text{C}/^{13}\text{C}$ KIEs is given in Ref. 7) based on Bigeleisen's treatment of the relationship between $^{12}\text{C}/^{14}\text{C}$ and $^{12}\text{C}/^{13}\text{C}$ KIEs.⁸

Theoretical model calculations of $^{11}\text{C}/^{14}\text{C}$ and $^{12}\text{C}/^{14}\text{C}$ KIEs using the program BEBOVIB⁹ were performed and the results together with the experimental data give some indication of the position of the transition state (TS) along the reaction coordinate. The relationship between the different carbon isotope effects was also calculated from the theoretical KIEs.

EXPERIMENTAL

General. ^{11}C was obtained as [^{11}C]carbon dioxide in a lead-shielded glass tube, containing 4 Å molecular sieves, at the tandem van der Graaff accelerator at the University of Uppsala. The tube was transported to the chemical laboratory and was connected to a special reaction vessel. All work with ^{11}C was performed behind lead shields.

A proportional regulating thermostat (HETO) with a regulating accuracy of $\pm 0.005^\circ\text{C}$ was used. The temperature of the water in the thermostat was measured by a calibrated thermometer with an accuracy of $\pm 0.01^\circ\text{C}$. The temperature never deviated more than $\pm 0.01^\circ\text{C}$ during a kinetic run. All glassware used was cleaned in chromic acid and then rinsed with water, 2 M sodium hydroxide solution, 2 M hydrochloric acid and distilled water. The glassware was dried at 130°C overnight and then kept in a desiccator, over bluegel, under a nitrogen atmosphere. The syringes were washed with ethanol, 2 M sodium hydroxide solution, 2 M hydrochloric acid, distilled water, ethanol and finally diethyl ether, dried with a hot-air blower and before use repeatedly rinsed with the solvent to be used.

HPLC analyses were performed on a Hewlett-Packard 1084 instrument with a β^+ -flow detector¹⁰ in series with the diode-array UV detector of the instrument. The HPLC system was equipped with a fraction collector (Hewlett-Packard 79825 A) slightly modified by removing the PTFE insert. The HPLC

analyses were performed on a column (200×4.6 mm i.d.), packed with Supelco ODS-1 ($10\ \mu\text{m}$). The mobile phase was 0.05 M ammonium formate (pH 3.5) – methanol (50:50, v/v), at an isocratic flow-rate of $2.00\ \text{ml}\ \text{min}^{-1}$. The injection volume was varied from 3 to $20\ \mu\text{l}$. The wavelength of the UV detector was 254 nm, using 430 nm as the reference.

The radioactivity counting was performed using an LKB 1214 liquid scintillation counter. The energy windows for the ^{11}C ($E_{\text{max}} = 0.98\ \text{MeV}$) and ^{14}C ($E_{\text{max}} = 156\ \text{keV}$) measurements were set to 1–2000 keV (window 5–1024) and 1–160 keV (window 5–650), respectively. The counting time was 1 min and 5–60 min for the ^{11}C and ^{14}C measurements, respectively. A ^{14}C -quench calibration graph was obtained by plotting quench parameter values against the counting efficiency. The different calibration points were determined by adding small volumes (0.25–1 ml) of the HPLC mobile phase to a scintillation bottle containing a ^{14}C standard capsule (internal standard kit for liquid scintillation counting, LKB) and 16 ml of scintillation liquid (Zinsser Quickszint 1). The volume of mobile phase added was chosen so that the quench parameter values of the ^{14}C standard bottle became close to the values of the sample fractions from the kinetic experiments.

Materials *N,N*-Dimethyl-*p*-toluidine (DMpT) (EGA-Chemie) was purified, (purity >99% checked by gas chromatography), according to a literature procedure.¹¹ Methanol (Riedel de-Haën, Spectranal grade) was distilled and dried over 3 Å molecular sieves and kept under nitrogen. Tetrahydrofuran (THF) (Merck, p.a. grade) was freshly distilled from sodium–benzophenone under a nitrogen atmosphere. Lithium aluminium hydride (LAH) (Merck, zur Synthese grade) (1-g tablets, purity >98%) was used. A solution of LAH in THF was prepared by cutting a LAH tablet under a nitrogen atmosphere and adding ca 0.2 g to 10 ml of THF in a flask which was capped with a septum and kept under a nitrogen atmosphere overnight, the concentration of the solution being ca 1 M. Concentrated hydriodic acid was distilled at atmosphere pressure (b.p. 126.5 – 127.5°C).

HPLC solvents were 0.05 M ammonium formate (pH 3.5) and methanol (FSA, HPLC grade). The mobile phase in the experiments had the following composition: (A) 0.05 M ammonium formate (pH 3.5) and (B) 0.05 M ammonium formate (pH 3.5) – methanol (20:80, v/v); the ammonium formate buffer

contained 0.03 M tetramethylammonium hydrogensulphate, acting as an amine modifier in the HPLC system. The HPLC element gradient was as follows: at time 0–2 min, 20% B; 2–5 min, 20–80% B; 5–6 min, 80% B; and 6–7.5 min, 80–20% B.

^{14}C Methyl iodide. ^{14}C Methyl iodide was purchased from Amersham (1.85 MBq, 2.0 GBq mmol^{-1}), at delivery 98.8% pure. The ^{14}C methyl iodide was delivered in a break-seal ampoule; the opened glass joint part was washed with 2 M sodium hydroxide solution, 2 M hydrochloric acid and absolute ethanol. A clean, dry, PTFE-covered magnetic stirrer bar was carefully inserted into the break-seal hook and the ampoule was connected to a class vacuum line. The line was connected to the vacuum pump and vacuum (0.1 mmHg) was applied for about 30 min while the line was heated with a hot-air blower. The vacuum line was filled with dry nitrogen and evacuated repeatedly, three times. To a 10 ml flask, connected to the vacuum line, 1 ml of dry methanol was added, under nitrogen, and the flask was cooled to -100°C . Finally the vacuum line was evacuated and the valve to the pump was closed. The stirrer bar was used as a hammer on the break-seal hook and by lifting and releasing the stirrer bar by means of an external magnet the hook was smashed. The ampoule was heated with a hot-air blower to facilitate the distillation of the ^{14}C methyl iodide into the cold methanol. After about 3 min the line was filled with nitrogen and the flask containing the solution was capped with a septum and kept in a desiccator under nitrogen, over bluegel, in a freezer.

^{11}C Methyl iodide. ^{11}C methyl iodide was synthesized using ^{11}C carbon dioxide by a three-step synthesis route.¹² The ^{11}C carbon dioxide was released from the lead-shielded glass tube containing molecular sieves during heating and transferred in a stream of nitrogen to the reaction vessel in which it was trapped in 0.7 ml of 0.5 M LAH in THF. After evaporation of the THF the residue was treated with concentrated hydriodic acid and the reaction mixture was heated to reflux, during which the ^{11}C methyl iodide formed was distilled off and transferred in a stream of nitrogen, through a drying tower, to a vial containing 600 μl of methanol, cooled to -72°C .

Kinetic procedure. A DMpT solution in methanol, concentration 1.0–1.2 M and volume 0.5–2 ml for different experiments, was prepared and thermostated before the ^{11}C radioactivity had arrived at the laboratory. When the ^{11}C methyl iodide synthesis was complete, 500 μl of the methanol solution, containing the ^{11}C methyl iodide, was transferred via a syringe to a vial containing *ca* 5 μl of ^{14}C methyl iodide solution per 100 μl of final reaction solution, and this mixture was thermostated. The DMpT solution was

added to the methyl iodide solution via a thermostated syringe. The reaction clock was started and, after mixing the resulting solution vigorously for a few seconds, the solution was distributed to 2–16 reaction vials (three different sizes were used: 350 μl , 1.5 ml and 2 ml, depending on the volume used) with a thermostated syringe. The vials were rapidly capped with gas-tight septa and placed in the thermostat. The quenching of a reaction point was performed by two different procedures. One procedure was by addition of 85% phosphorous acid (10 μl per 100 μl of reaction solution) and subsequent, immediate, cooling to -72°C . The other quenching was performed by direct injection of the reaction mixture into the HPLC system, recording the reaction time at the moment of injection. After quenching of the reaction mixture by the acid quenching procedure, this mixture was also injected into the HPLC system. The reactant (methyl iodide) and product (the quaternary amine) were separated and collected in scintillation bottles containing 16 ml of scintillation liquid. The ^{11}C radioactivity of the fraction bottles was immediately measured by liquid scintillation counting. The fractions containing least radioactivity were measured first. When the ^{11}C had disintegrated, the ^{14}C radioactivity of the fraction was measured.

RESULTS AND DISCUSSION

The concentrations used in the methylation were 0.65–0.7 M with respect to DMpT and 0.5–1 mM with respect to methyl iodide. The amine concentration was thus in a *ca* 1000-fold excess over the methyl iodide concentration, making the methylation a pseudo-first-order reaction. The KIE was calculated by two different procedures, one of which was determination of the ratio of the slopes from the plot of $-\ln(1-f)$ versus reaction time for ^{11}C and ^{14}C , respectively, where f is the

Table 1. Results of $^{11}\text{C}/^{14}\text{C}$ KIE experiments of the reaction of DMpT with methyl iodide in methanol at 30°C

Slope ratio ^a KIE $\pm s^c$	Point ^b KIE $\pm s^d$	No. of points
1.183 \pm 0.033	1.193 \pm 0.006	9
1.217 \pm 0.010	1.221 \pm 0.008	16
1.215 \pm 0.024	1.214 \pm 0.007	14
1.192 \pm 0.025	1.188 \pm 0.007	10

^a Calculated as the ratio of the slopes of the ^{11}C and ^{14}C lines from the plots of $-\ln(1-f) = F(t)$.

^b Calculated as the mean value of the KIEs in each point, according to equation (4).

^c The standard deviation of the slopes were calculated by the equation $s = s_{y/x} / [\sum (x_i - \bar{x})^2]^{1/2}$, where x_i is the reaction time for point i and \bar{x} is the mean of the x_i values. $s_{y/x}$ is given by the equation $s_{y/x} = [\sum (y_i - \bar{y})^2 / (n - 2)]^{1/2}$, in which y_i is the value of $-\ln(1-f)$ in point i and \bar{y} is the point on the calculated regression line, and n is the number of points. In the calculations of the standard deviation of the slope ratio the ordinary rules of propagation of error were used.

^d The standard deviation of the point KIEs were calculated as usual.

fraction reaction, calculated as

$$f = P/(P + R) \quad (1)$$

where P and R are the corrected radioactivity counts per minute (cpm) values of the product and reactant fractions, respectively. The KIE was also calculated as the mean value of the KIE in each point [calculated according to equation (4)]. The results of the kinetic experiments are summarized in Table 1.

At 30 °C the $^{11}\text{C}/^{14}\text{C}$ KIE was 1.202 ± 0.008 ($n = 4$)

calculated as the ratio of the ^{11}C and ^{14}C slopes from the plots of $-\ln(1 - f) = F(t)$ and 1.204 ± 0.008 ($n = 4$) calculated as the mean point-KIE.

KIE measurements were also performed at 15, 20, and 40 °C. The accuracy of these KIEs was poorer than those measured at 30 °C, probably owing to temperature effects at the injection moments, and no conclusion about the temperature dependence of the KIE could be made.

In the earlier communication² k_{11}/k_{14} was deter-

Table 2. Kinetic results from one experiment of the reaction of DMPt with labelled methyl iodide in methanol at 30 °C

Point No., fraction	^{14}C data			^{11}C data				Point KIE ^c
	Cpm	$SQP(E)^a$	$-\ln(1 - f)^b$	Cpm	ETIME ^c	Cpm _{corr} ^d	$-\ln(1 - f)^b$	
1,1	2062.0	416.7	0.12649	217450	3431	1.5106	0.13926	1.2166
1,2	15324	422.2		93969	8522	10.109		
2,1	2713.0	420.6	0.22319	253542	3520	1.8534	0.23479	1.1115
2,2	10857	418.9		62622	8612	7.0033		
3,1	7370.9	419.1	0.28313	121778	6872	5.6972	0.33209	1.2249
3,2	22551	415.7		161720	8152	14.465		
4,1	5472.3	419.6	0.37814	295028	4684	4.1462	0.43867	1.1981
4,2	11920	416.9		88205	8063	7.5297		
5,1	6641.8	419.8	0.46795	115169	6782	5.1307	0.55725	1.2222
5,2	11142	416.9		84649	7970	6.8788		
6,1	6050.6	419.2	0.55259	268052	5037	4.5850	0.65311	1.2082
6,2	8209.4	416.6		70238	7702	4.9755		
7,1	6644.4	421.4	0.63291	240147	5415	5.0686	0.75551	1.2168
7,2	7535.2	417.5		225167	5325	4.4907		
8,1	7247.4	421.1	0.71638	58327	8241	5.5657	0.86065	1.2219
8,2	6933.7	416.7		215102	5234	4.0783		
9,1	7919.8	418.9	0.78009	60147	8330	5.9917	0.93422	1.2163
9,2	6707.6	416.8		215032	5144	3.8776		
10,1	8399.1	420.5	0.85182	46224	8984	6.3124	1.0205	1.2152
10,2	6257.1	417.4		139726	5775	3.5570		
11,1	8813.2	419.2	0.98138	44263	9165	6.5795	1.1704	1.2073
11,2	5284.0	418.9		110836	5865	2.9595		
12,1	9439.5	421.9	1.0877	49550	9073	7.0499	1.2981	1.2068
12,2	4799.7	421.1		81559	6229	2.6481		
13,1	9529.8	422.3	1.0905	45600	9255	7.0524	1.3072	1.2121
13,2	4825.1	421.4		76946	6317	2.6160		
14,1	10345	422.0	1.2119	47687	9344	7.6889	1.4577	1.2149
14,2	4388.4	419.5		40453	7328	2.3328		
15,1	10352	421.8	1.2344	45780	9436	7.6941	1.5075	1.2332
15,2	4255.8	417.7		18917	8792	2.1886		
16,1	18856	420.0	1.4504	75573	9613	13.691	1.7399	1.2096
16,2	5775.7	419.8		24258	8881	2.9149		$\langle x \rangle^f = 1.204$
$0^{14}\text{C},1$	400.75	418.2	0.01269					
$0^{14}\text{C},2$	31381	420.4						
$0^{11}\text{C},1$					1383	116	0.000345	
$0^{11}\text{C},2$				35449	8434	4.2250		
$\infty^{14}\text{C},1$	82.3	403.4	5.389					
$\infty^{14}\text{C},2$	17942	404.4						

^a $SQP(E)$ = scintillation quench parameter.

^b f is the fraction of reaction, calculated according to equation (1).

^c ETIME = elapsed time from start of the counting.

^{d,e} Cpm_{corr} and point KIE were calculated according to equation (3) and (4), respectively (see below).

^f In the calculations of the mean point KIE value, $\langle x \rangle$, points 2 and 15 were rejected by the Q -test.

mined to 1.23 ± 0.04 (the standard deviation of the worst case). This value is larger but not significantly different, within the standard deviation reported, from the new value of 1.202 ± 0.008 ($n = 4$). The $^{12}\text{C}/^{14}\text{C}$ KIE value was earlier determined to be 1.117 ± 0.011 at 48.5°C . The $^{12}\text{C}/^{14}\text{C}$ KIE value was calculated⁷ to be 1.122, using the equation

$$r \equiv \ln(k_{11}/k_{14})/\ln(k_{12}/k_{14}) \approx 1.6 \quad (2)$$

where k_i are the rate constants for the isotopic reactions. This is in good agreement with the experimentally determined k_{12}/k_{14} value.

Because of the large amount of data for each experiment, only the results of one, representative, experiment are presented in Table 2.

Corrections of the ^{11}C cpm values were performed using the equation $Z = X - (Y + B)$, where B is the background cpm value of the ^{11}C measurements, measured next to the fraction, Y is the ^{14}C cpm value, X is the total cpm value and Z is the ^{11}C cpm value. The half-life corrections were then made according to the equation

$$\text{cpm}_{\text{corr}} = Z/[0.5 \exp(t/t_{1/2})] \quad (3)$$

where cpm_{corr} is the decay-corrected ^{11}C cpm value, t is the elapsed time (ETIME), in seconds, and $t_{1/2}$ is the ^{11}C half-life. The ^{11}C cpm_{corr} and ^{14}C cpm values for the two fractions of one point was used to calculate $-\ln(1-f)$. The values of $-\ln(1-f)$ were plotted

versus reaction time for the ^{11}C and ^{14}C reactions (Figure 1).

The point KIEs were calculated from the equation

$$\text{point KIE} = {}^{11}(Q_i - Q_0)/{}^{14}(Q_i - Q_0) \quad (4)$$

where the superscripts 11 and 14 denote that the subtraction in the parentheses applies to ^{11}C and ^{14}C , respectively, Q_i is the $-\ln(1-f)$ value for point i and Q_0 is the $-\ln(1-f)$ value for the reactant at $t = 0$. This was a correction for radioactive impurities.

From this graph it can be seen that the ^{11}C and ^{14}C values of $-\ln(1-f)$ usually deviate from the calculated regression line in the same way relative to each other. Hence there is a random error contribution to the slope ratio KIE, which is not present in the point KIE calculation. This random error arises from the HPLC fractionation due to peak broadening or too short an equilibration time between two injections. For the point KIE calculation careful analyses of the reactant solution are required since corrections for the 'zero point' are made in each point.

The only detectable side-product in the ^{11}C -labelling synthesis was [^{11}C]methanol, usually less than 0.5%. The methanol concentration showed no increase during a kinetic run, i.e. there was no measurable hydrolysis side-reaction. The concentration of labelled methanol in the [^{14}C]methyl iodide solution increased with storage time, and the iodide was redistilled when the solution contained more than 2% of [^{14}C]methanol. In

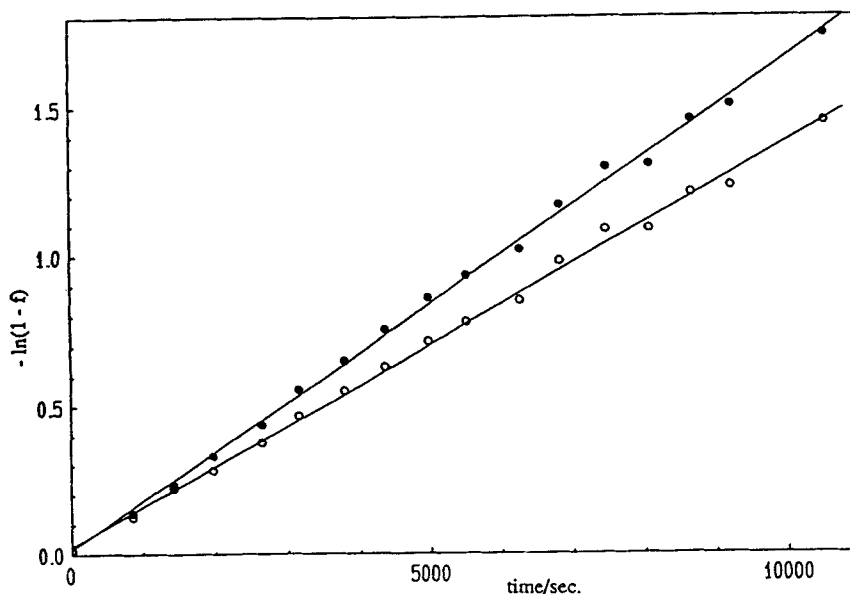


Figure 1. Plots of $-\ln(1-f)$ versus reaction time for the experiment presented in Table 2 for the reaction of DMpT with labelled methyl iodide in methanol at 30°C (●) ^{11}C reaction; (○) ^{14}C reaction

Figure 2 the UV and radio-chromatograms of a typical analysis are shown.

The $^{11}\text{C}/^{14}\text{C}$ KIE method is based on HPLC separation. The reason for choosing HPLC was that work with ^{11}C demands rapid procedures, and HPLC fits this criterion and permits reproducible fraction collection.

In the reaction studied, methyl iodide and the quaternary amine (QA) had to be separated and fractionated in small volumes, and thus narrow HPLC peaks were required. The fraction volumes were limited by the requirement that the ratio between HPLC mobile phase and scintillation liquid in the scintillation bottles had to be low, ensuring a high counting efficiency and reproducible measurements. The solution had a high concentration (0.7 M) of a tertiary amine (DMpT) which led to peak broadening and other problems. These problems could be solved by use of amine modifiers. Quaternary ammonium salts, such as tetramethylammonium hydrogensulphate, made the separation possible with narrow peaks (see Figure 2). Concentrations in the range of 5–30 mM were used in the mobile phase.

The quenching of the reaction was performed by two different procedures: by addition of acid or by direct HPLC injection. A series of experiments were performed in which the reaction solution was mixed and portioned into 10–15 vials which were capped and thermostated. The reaction in every second vial was quenched by addition of acid and the others were quenched by direct injection, at the same time intervals as in a normal kinetic run. No significant difference between the magnitude of the standard deviation from the slope ratio KIE or the point KIE, relative to normal experiments, was detected. There was also no difference in the standard deviation between the two quench procedures.

Methyl iodide is volatile (b.p. 42.4°C), and exper-

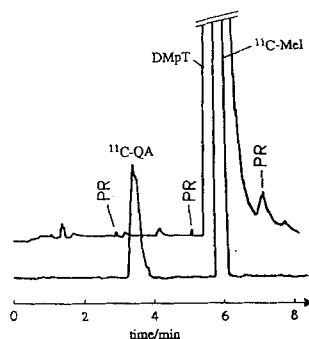


Figure 2. Radio-chromatogram (bottom) and UV chromatogram (top) for the HPLC fractionation of [^{11}C]QA (product, quaternary amine) and [^{11}C]methyl iodide. the large peak in the UV chromatogram is DMpT. Pr indicates the moment of fractionation

iments were performed to test if the reaction was affected by changes in the gas-phase volume in the vials and if there was any leakage through the septa. A 1 mM solution of methyl iodide in methanol was prepared in a vial and capped. The septum was then penetrated five times with the HPLC injector needle during analyses. The vial was then kept at 30°C for 2.5 h and the contents were again analysed five times. No change in the methyl iodide concentration was detected. Experiments were also performed to test if the reaction was dependent on the volume of the gas phase. After mixing the reaction solution, it was delivered into 10–15 vials (300 μl). To half of these 100 μl of solution were added and the other half were filled to the brim. The vials were then capped and thermostated. At time intervals vials were withdrawn, alternately a full vial and a part-full vial, and the contents were injected into the HPLC system, quenching being performed by direct injection. There was no measurable gas-phase volume dependence of the reaction.

In another experiment, the reaction was quenched in five vials with acid when the reaction had proceeded to *ca* 20% conversion and another five vials were quenched at *ca* 50% conversion. The quenching of the five vials was performed within 30 s. The standard deviation of the five points was the same, relative to each other and relative to a kinetic experiment performed in the usual way. The standard deviation of the results of experiments run in different vials was thus the same as for the one-pot procedure. One must, of course, bear in mind that the statistical tests discussed above were made on the basis of only 5–7 points in all the experiments, which may seem poor, but the short half-life of ^{11}C sets the restrictions on tests such as these.

Three different methods of measuring ^{11}C radioactivity were compared with each other: liquid scintillation, Čerenkov counting and sodium iodide crystal counting. [The sodium iodide crystal is used as a solid scintillator for gamma-ray detection. The gamma-rays from ^{11}C are produced by a positron–electron annihilation and have an energy of 511 keV. In liquid scintillation counting the radioactive substance is dissolved in a solvent which contains an organic scintillator, i.e. the source is intimately mixed with the detector. In liquid scintillation counting beta-particles are measured, either β^- , as for ^{14}C , or β^+ , as for ^{11}C . The maximum energies of the beta-particles for ^{14}C and ^{11}C are 0.156 and 0.98 MeV, respectively. In Čerenkov counting no scintillator or organic solvent is added to the aqueous sample. The counting is performed using a liquid scintillation counter and measures the scintillations produced by the Čerenkov effect. The observed radioactivity depends on the sample volume. This method can only be applied to high-energy beta-emitters (>0.7 MeV), hence ^{14}C cannot be measured.]

Samples were prepared by HPLC fractionation of a

[^{11}C]methyl iodide solution and collecting the methyl iodide fraction (4 ml) in an empty scintillation bottle. The radioactivity of this solution was measured by the solid scintillation method (sodium iodide crystal) and by the Čerenkov method. Then 16 ml of scintillation liquid were added to the bottle and the radioactivity was measured by the liquid scintillation method and by the solid scintillation method. The relative sensitivity of ^{11}C counting was found to be 100%, 40% and 25% for the liquid scintillation, solid scintillation and Čerenkov counting, respectively. ^{11}C measurements by these three counting methods were performed during full kinetic experiments. The Čerenkov method differed from the others, giving significantly different values of fraction reaction and a larger standard deviation. When the radioactive fractions were collected in scintillation bottles containing scintillation liquid, there was no measurable difference in the comparison between the solid scintillation and the liquid scintillation method within a relative standard deviation of 1%.

Chemical quenching, originating from the fact that the samples in the liquid scintillation counting contained 20% HPLC eluent, was detected. The difference in mobile phase composition (due to the gradient) between the reactant and product fractions did not give rise to different quenching, nor did the DMpT concentrations in the measured fractions have any effect on the quenching, although DMpT quenched the sample solution at higher concentrations (about 100 times higher). The samples contained both ^{11}C and ^{14}C radioactivity, and two different methods were used to measure the respective magnitudes. In Figure 3 the energy spectra of the two isotopes are shown. The ^{14}C radioactivity was always measured in channel C (window 5–650) (see Figure 3) when the ^{11}C radioactivity had decayed (usually the next day). The ^{14}C radioactivity in the fractions was usually 500–10 000 cpm. The ^{11}C radioactivity, in the first method, was measured in channel A (window 5–1024) and had to be corrected for the ^{14}C contribution before half-life corrections (see Table 2).

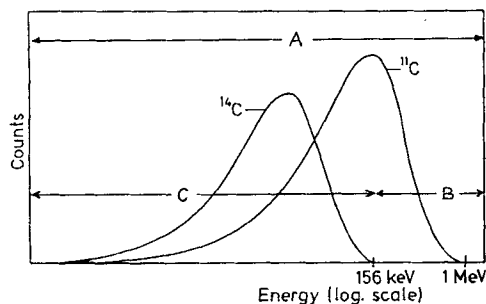


Figure 3. Beta-spectra of ^{11}C and ^{14}C measured by liquid scintillation counting. A, B and C are the different energy windows used in radioactivity counting

In the second method ^{11}C was measured in channel B (window 650–1024) and thus the measured radioactivity did not have to be corrected for ^{14}C radioactivity contribution. The first method was the one of choice since the second method was not as accurate and the counting efficiency of the first was higher. The dead-time in liquid scintillation counting is dependent on the total radioactivity summed over all the windows and since channel B only measures ca 21% of the total ^{11}C disintegrations a long dead-time was obtained for low activities, compared with the measurements by the first method. Dead-time corrections made by the instrument used were found, with our own experiments, to be accurate even at dead-times over 10%, but to be sure that the dead-time corrections used in the experiments were accurate no count values with a dead-time higher than 7% were used. At this dead-time the values were ca 250 000 and 50 000 cpm for the first and second methods, respectively. The standard deviation, defined as the square root of the counts, was then lower than half the standard deviation for the first method. A more serious drawback with the second method was that the percentage of disintegrations in channel B was decreasing with the radioactivity level. This effect was easily measured for a sample of high ^{11}C radioactivity. At 800 000 cpm the radioactivity in channel B was more than 22% of the total radioactivity. When the radioactivity had decreased to 100 000 cpm, the amount in channel B was only 20%. The first method should thus be used for ^{11}C measurements. An analysis of quenching in the ^{11}C measurements was not performed. The reason is that quenching is greater for radionuclides with low than for those with high beta energy and, since the ^{11}C maximum energy is about six times larger than that for ^{14}C , the quenching in the ^{11}C measurements was presumed to be smaller or of the same order. To obtain a quench curve for short-lived radioactive isotopes is also difficult. The samples contained two radionuclides and their relative amounts in the solution were changed very quickly, making it difficult to produce such a quench curve.

The quench parameter $SQP(E)$ is derived by an external standard method and calculated by the instrument. The quench curve, derived from plotting the counting efficiency values versus the $SQP(E)$ values, was analysed and the standard deviation was calculated around the quench parameter region of the samples. The corrections were not large; for the experiment presented in Table 2 it was 418.5 ± 1.1 , representing a counting efficiency of $91.4 \pm 0.2\%$. Note that the standard deviation of the quench parameter was smaller than that of the ^{14}C radioactivity counting (less than 0.5%). In the present method, the error of quenching was low because the difference in HPLC eluent composition in the reactant and product fractions did not affect the quenching and because the eluent volumes were equal to each scintillation bottle. For a sample

Table 3. Structural parameters^a and force constants^b for reactant and TS models

Bond	Reactant		Transition state	
	r_i	F_{ii}	r_i	F_{ii}
C—I	2.139 ^c	2.31 ^c	2.139 - 0.3(ln n_{CI})	2.31 n_{CI} ^c
C—H _{1,2,3}	1.094 ^d	4.97 ^d	1.094 ^d	4.97 ^c
C ₁ —N			1.48 ^d - 0.3(ln n_{CN})	5.10 n_{CN} ^d
N—C _{2,3,4}			1.48 ^d	5.10 ^d

Angle bend	Reactant		Transition state	
	Angle	F_α	Angle	F_α
I—C—H _{1,2,3}	109.47	0.54 ^e	Θ	$g_\alpha(n_{CI}n_{CH})0.54^e$
H _i —C—H _j	109.47	0.55 ^d	ϕ	$g_\alpha(n_{CH}n_{CH})^{1/2}0.55^d$
N—C—H _{1,2,3}			180 - Θ	$g_\alpha(n_{CN}n_{CH})0.70^d$
C _{2,3,4} —N—C _{2,3,4}			109.47	1.035 ^f
C ₁ —N—C _{2,3,4}			109.47	$g_\alpha(n_{CN}n_{NC})1.035^f$
C ₂ —N—C ₁ —H ₁ (tors.)			180	0.072 ^d

^a The bond angles and distances are given in degrees and ångströms, respectively.

^b Force constants are given in mdy \AA^{-1} (stretching) and mdy $\text{\AA} \text{rad}^{-2}$ (bending and torsional).

^c Ref. 13b.

^d Ref. 13e.

^e Ref. 15.

^f Ref. 16.

measured several times the standard deviation was more than half the standard deviation for all samples in an experiment, implying that more than half of the spread in counting efficiency originates from the instrument. As a result, the standard deviation of the counting efficiency of a full experiment was less than 0.1%. Owing to this, no corrections were performed on the ¹⁴C cpm values regarding counting efficiency.

Theoretical model calculations

Theoretical model calculations of primary carbon KIEs for S_N2 reactions have previously been performed by several workers.¹³ The present calculations are basically performed as in our work³ on the reaction of labelled methyl iodide with hydroxide ion, in which a more detailed account on the calculation procedure is given.

Calculations were carried out on the BASF 7/68 computer at the computer centre of the University of Uppsala using the program BEBOVIB-IV.⁹

Geometrical model.

For the reactant a five-atom full model was used. The geometry was tetrahedral around C and normal single bond values were taken from the literature (see Table 3). For the transition state a nine-atom cut-off model¹⁴ was used, according to Figure 4. The C—H and N—C_{2,3,4} bond orders (n_{CH} and n_{NC} , respectively) were kept at 1.0.

The bond orders for the rupturing C—I and the

forming C—N bonds (n_{CI} and n_{CN} , respectively) were varied between 1.0 and 0.0 under the assumption of conserved total bond order to C in the transition state models, i.e. $n_{CI} + n_{CN} = 1.0$. The angles Θ and ϕ were given by the relationships

$$\Theta = 70.53 + 38.94n_{CN}$$

$$\phi = \cos^{-1}(1 - 1.5 \sin^2 \Theta)$$

ensuring the expected Walden inversion in going from reactant to product.

Force constants

A simple valence force field was used for both the reactant and the TS models except for the interaction force

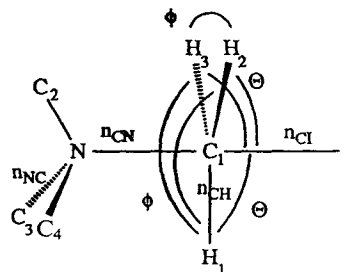


Figure 4. The TS structure for the reaction of DMpT with methyl iodide. n_{CI} and n_{CN} are the bond orders for the leaving group (I^-) and nucleophile (DMPT) to the central carbon atom, respectively, and ϕ and Θ are bond angles

constants required to generate the reaction coordinate frequency in the TS. The force constants of the reactant model were standard values taken from the literature (see Table 3). The force constants of the TS model were calculated from the bond orders and the standard force constants through a set of empirical relationships, in the customary way.⁹

Reaction coordinate generation.

Three different reaction coordinate models were employed.^{13c} In the simplest model (A), the stretching coordinate for the breaking C—I bond was coupled to the stretching coordinate for the forming C—N bond (coupling constant $a_{ij} = 1.1$).

A more pronounced Walden inversion was obtained in a model (B) where further interaction constants were used to couple the C—N stretching mode to each of the H—C—N bending modes and the C—I stretching mode to each of the H—C—I bending modes (for all additional couplings the constant 0.3 was used). In the third reaction coordinate model (C), the same Walden inversion was produced as in model B for the symmetric TS but the contribution of this motion was diminished for more reactant- or product-like transition states. This was accomplished by the use of the equation proposed by Yamataka and Ando:^{13e}

$$a_{\text{str, bend}} = a_{\text{str, bend}}^s (1 - \Delta n)^2 \quad (5)$$

where $\Delta n = |n_{\text{CI}} - n_{\text{CN}}|$ and $a_{\text{str, bend}}^s = 0.3$ is the coupling constant for the symmetric TS model.

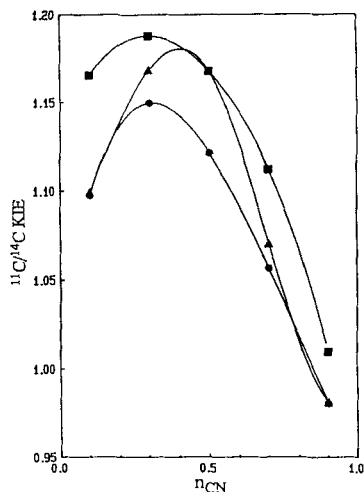


Figure 5. Calculated $^{11}\text{C}/^{14}\text{C}$ KIE versus bond order, n_{CN} , for the reaction of DMpT with methyl iodide at 30 °C. The calculations were performed for three reaction coordinate models, (●) A, (■) B, (▲) C; see text for explanation of the models. The top axis at 1.2, also represents the experimentally determined value

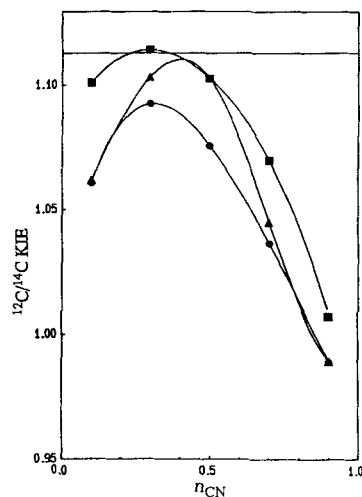


Figure 6. As Figure 5, but showing the calculated $^{12}\text{C}/^{14}\text{C}$ KIEs. Symbols as in Figure 5. The horizontal line represents the experimentally determined value

The results of $^{11}\text{C}/^{14}\text{C}$ and $^{12}\text{C}/^{14}\text{C}$ KIE calculations are presented in Figures 5 and 6, respectively. The KIEs show the same type of bell-shaped behaviour as has been obtained in previous calculations. Experimental results are represented as horizontal lines. For the $^{12}\text{C}/^{14}\text{C}$ KIE, the experimental value show agreement with model B at bond order 0.2 and 0.4 for the forming C—N bond. The calculated $^{11}\text{C}/^{14}\text{C}$ KIE values are lower than the experimental value but the best accordance is for model B at bond order 0.3 for the forming C—N bond. Hence these results indicate a fairly reactant-like TS.

The relationship between the different carbon KIEs, i.e. the r values as defined by equation (2), obtained from the theoretically calculated KIEs show good agreement with the estimated value of 1.6. This is in fair accordance with the experimental r value of 1.66. Interestingly, in the $\text{S}_{\text{N}}2$ reaction of methyl iodide with hydroxide ion,³ a discrepancy between the experimental and theoretical relationship has recently been observed.

CONCLUSION

The $^{11}\text{C}/^{14}\text{C}$ method has so far been used for determination of the primary $^{11}\text{C}/^{14}\text{C}$ KIE for the reaction of hydroxide ion with methyl iodide.³ A secondary $^{11}\text{C}/^{14}\text{C}$ KIE has been determined for the base-catalysed prototropic rearrangement of 1- to 3-methylindene¹⁴ and the β -tyrosinase-catalysed reaction of L-tyrosine to pyruvic acid and phenol has also been studied.¹⁷ The accuracy of the results from these experiments was relatively good and the KIE measurements were repro-

ducible. Hence this method has proved useful in different kinds of carbon KIE studies.

Finally, we would like to quote Melander and Saunders in Ref. 1: 'Liquid scintillation counting has acquired among many chemists an undeserved reputation for being imprecise and unreliable. This impression arose in part from deficiencies of early instruments.... Modern scintillation counters are actually remarkably precise, reliable and easy to use.'

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